DIFFERENTIATION OF WOOD-DECAYING FUNGI BY THEIR REACTIONS ON GALLIC OR TANNIC ACID MEDIUM [1]

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INTRODUCTION

In a study of only a few wood-decaying fungi Bavendamm [3] found that those species causing white decay formed a dark diffusion zone or "corona" under the fungus mats when grown on media containing small amounts of gallic or tannic acid, whereas those causing brown checked decay gave no such reaction. The brown diffusion zone was considered to be the result of oxidation of the acids, and hereafter this reaction will be referred to as "oxidase" reaction. Bavendamm also pointed out the effect of various concentrations of the acids on growth of the several species and suggested the use of such reactions for identification purposes should they be found consistent for a greater number of species.

Campbell [4] recorded the oxidase reactions of species of *Fomes* on tannic acid medium and found them to be consistent with Bavendamm's prediction.

It is the purpose of the present paper to give results of repeated tests of a greater number of species, representing several families and numerous genera, mostly of the Hymenomycetes. The above-mentioned tests have been used to assist in developing a method of identifying pure cultures of the wood-decaying fungi. Many such fungi have been isolated from decay in living trees on which no sporophores occurred. Such a test as the oxidase reaction, which separates the species into two distinct groups, is, when combined with numerous macroscopic and microscopic characters, very useful. The fact that the media used for the oxidase test also have a toxic effect on some species and not on others makes them of even greater value for identification purposes.

Growth rates, as well as oxidase reactions, of the fungi have been recorded on media of only one concentration, namely 0.5 percent of gallic or tannic acid added to plain malt agar as recommended by Bavendamm, although it is realized that other concentrations might give additional information of value.

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[3] Bavendamm, W. Über das vorkommen und den nachweis von oxydasen bei holzzerstörenden pilzen. Zuschr. Pflanzenkrank. u. Pflanzenschutz 38: [257]–276, illus. 1928.
[4] Campbell, W. A. the cultural characteristics of the species of fomes. Bull. Torrey Bot. Club 65: 31–69, illus. 1938.

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SOURCE OF CULTURES

The cultures of the different fungi, the majority of which are wood-decaying, used to determine the oxidase reaction of the various species, were those previously identified and maintained in a collection by the Division of Forest Pathology.[5] Most of the isolates were from tissue of sporophores that had been carefully identified, many of them by L. O. Overholts, or from incipient decay with which identifiable sporophores were associated. Since isolates from these two sources react alike in culture, no indication is given in the list as to what proportion of the cultures used for each species were from sporophores. A few may not be definite wood decayers, but since they were associated with dead or decaying wood, they are included to make the study as complete as possible.

METHODS

The oxidase test is made by growing the fungus to be tested on malt agar to which gallic or tannic acid has been added. Gallic acid medium was prepared by the addition of 0.5 percent of gallic acid (Mallinckrodt, U. S. P. crystallized) to malt agar (1.5 percent of Difco malt with 2 percent of agar). Twenty grams of powdered agar and 15 g of Difco malt were dissolved in 850 cc of water in a 2-liter flask. One hundred and fifty cubic centimeters of water was placed in a separate flask. The dissolved malt agar and the flask of water were autoclaved for 20 minutes at a pressure of 15 pounds. Five grams of gallic acid was dissolved in the flask of sterilized water after removal from the autoclave. Heating gallic or tannic acid with agar causes hydrolysis of the agar; therefore the two cannot be autoclaved together. When the malt agar had cooled so that it could be handled without burning the hands, the gallic acid solution was added and thoroughly mixed. The resulting gallic acid medium was then quickly poured into 9-cm Petri dishes, about 35 cc to each dish. The dishes were spread in a single layer to insure rapid cooling. Gallic acid does not change the color of malt agar. Tannic acid medium was prepared in a similar manner, using 0.5-percent tannic acid (Mallinckrodt, U. S. P., powdered). This medium has a milk-white appearance.

In order to determine the effect of old and young mycelium on gallic and tannic acid media, two series of tests were made in which 1-year-old cultures and 2-month-old cultures, respectively, were used. Cultures of these different ages were used to see whether the older cultures had accumulated oxidases, which might produce stronger reactions. Results indicated that age of cultures beyond 2 months made little difference in reaction or rate of growth, as long as the cultures were still vigorous. Reactions are reported as averages, while in the case of growth the usual range in diameter of mat is given.

Gallic and tannic acids appear toxic to many fungi, and conclusive results seemed to depend upon the growth of the fungus on the media. For this reason large pieces of inoculum, 4 to 6 mm square, with as

[5] A number of the cultures in the reference collection were contributed by L. O. Overholts, Irene Mounce, Clyde Christensen, and Ray R. Hirt.

much fungus material as possible, were placed, mycelium down, on the agar and pressed gently in order to make close contact. When working with slow-growing fungi, from one to three inoculations of the same species were made in a single dish. Several commercial brands of gallic and tannic acids were tested to determine whether different sources of these acids would give comparable results. In general, the oxidase reactions were consistent but the growth rates varied considerably. Because of evident differences in commercial gallic and tannic acids, when comparing growth and to a lesser extent oxidase reactions of different fungi, it is important to use acids from the same commercial sources.

All the fungi, with the exception of a few recent acquisitions were tested for the oxidase reaction at least three times over a period of 2 years. The reactions and, to a lesser extent, growth (table 1) have appeared relatively consistent.

Observations upon the intensity of reaction and growth of mycelium were made at the end of 7 days for cultures kept in diffused light at room temperature (about 25° C.). In doubtful cases the cultures were kept for an additional 7 days. Most fungi that reacted with the acid media gave conclusive results at the end of 7 days. However, a number produced strong diffusion zones at the end of 1 or 2 days.

RESULTS

REACTION AND GROWTH DATA

The appearance and relation of the brown diffusion zone to the fungus mat, as well as the time required for its development, varied widely with different species. The following system was used to record the reactions of the different species to gallic and tannic acid media:

—, Negative, no brown discoloration of the agar under or about the mat.[6]

+, Diffusion zone light to dark brown, formed under inoculum at center of mat and visible only from under side of dish. In case no growth takes place, a faint brown discoloration under the inoculum.

++, Diffusion zone light to dark brown, formed under most of mat but not extending to margin. Visible from under side only.

+++, Diffusion zone light to dark brown, extending a short distance beyond the margin of the mat and visible from the upper side.

++++, Diffusion zone dark brown, opaque, extending considerably beyond margin of fungus mat.

+++++, Diffusion zone very intense, dark brown, opaque, forming a wide corona about mat. Usually such intense reactions occur with species giving no growth on the medium, and are most common on gallic acid medium.

Usually the brown diffusion zone which formed under the fungus mat would conform to one of the foregoing types. Occasionally a species gave a reaction that did not definitely fall into any of these groups; in such cases special mention is made.

The species of fungi showed interesting growth differences on malt agar containing 0.5 percent of gallic or tannic acid. These differences were caused by the toxicity of the acids. The following system

[6] Certain negative reactors cause decolorization of the medium under the mat. This decolorized zone, which forms most distinctly on tannic acid medium, should not be confused with the brown diffusion zone of typical reactors.

was used to record growth data: 0, no growth; tr., trace, growth confined to inoculum; 10, 15, etc., colony diameters in millimeters. Since growth rates were taken at room temperatures and are variable, they are expressed as a range rather than as an average.

Analysis of growth and reaction data at 7 days showed that the different fungi tested on gallic and tannic acid media could be classified into 10 fairly distinct groups. The behavior group for each individual fungus is given in the last column in table 1. Since this classification is based on growth in relation to type of reaction, no attempt was made to classify those species giving both positive and negative reactions on the same medium. A description of the groups and the number of fungi in each are given on pages 689–693.

TABLE 1.—*Reaction and growth of wood-decaying fungi on gallic and tannic acid media, at the end of 7 days*

Fungus	Type of decay	Isolations	On gallic acid medium		On tannic acid medium		Growth and reaction group No.[1]
			Reaction	Growth	Reaction	Growth	
		Number		*Mm*		*Mm*	
Thelephoraceae:							
Coniophora cerebella Pers.	Brown	5	−	30–50	−	20–35	1
C. suffocata (Peck) Massee	do	2	−	45–60	−	15–20	2
Corticium confluens Fr.	White	2	+++	0	++++	Tr.	4
C. coeruleum (Schrad.) Fr.	do	1	[2]− to +	30	+	Tr.	9
C. galactinum (Fr.) Burt	do	2	+++++	Tr.	+++	20–40	6
C. hydnans (Schw.) Burt		1	+++++	0	+++	10	5
C. investiens (Schw.) Bres.	White	1	+++++	Tr.	+++	20	5
C. lividum Pers.	do	3	+++++	25–45	+++	20–30	7
Cutidia salicina (Fr.) Burt		1	+++	0	+++	Tr.	4
Hymenochaete agglutinans Ellis		2	++++	Tr.–30	+++	30–50	8
H. corrugata (Fr.) Lév.	White	1	+++++	15	+++	45	8
H. curisii (Berk.) Morgan	do	1	+++++	20	++++	20	7
H. rubiginosa Dick. ex Lév.	White pocket.	6	++++	Tr.–15	+++	Tr.–10	7
H. tabacina Sow. ex Lév.	White	5	+++++	20–35	++	20–50	7
Peniophora allescheri Bres.	do	1	++++	0	+++	Tr.	4
P. cinerea (Pers.) Cooke	do	2	+++++	Tr.	+++	35	6
P. coccineo-fulva (Schw.) Burt		1	++++	35	++	Tr.	9
P. gigantea (Fr.) Massee		1	[2]+	10	−	0	9
P incarnata (Pers.) Karst.		2	+++++	Tr.–20	++++	25–40	8
P. nuda (Fr.) Bres.	White	1	+++++	15	+++	35	8
P. pubera (Fr.) Sacc.	do	3	+++++	15–40	+++	20–60	7
Stereum albobadium (Schw.) Fr.	do	1	+++++	Tr.	+++	50	6
S. fasciatum Schw.	do	5	++++	15–40	+++	20–50	7
S. frustulosum (Pers.) Fr.	White pocket.	13	−	25–45	−	20–35	1
S. fuscum Schrad. ex Quél.	White	3	++++	Tr.–10	−	0	9
S. gausapatum Fr.	do	12	++++	10–25	+++	25–35	7
S. murrayi (Berk. and Curt.) Burt	do	6	++++	0	+++	Tr.–10	5
S. pini Fr.	do	2	+++++	Tr.	+++	20–40	6
S. purpureum Pers.	do	4	++++	0–tr.	+++	25–40	6
S. rameale Schw.	do	10	++++	20–50	+++	35–60	7
S. roseo-carneum (Schw.) Fr.	do	1	+++++	Tr.	+++	25	5
S. rugosiusculum Berk. and Curt.	do	1	+++	0	++	40	6
S. sericeum Schw.	do	1	++++	Tr.	+++	25	5
S. subpileatum Berk. and Curt.	White pocket.	10	[3]− to ++	10–15	−	10–20	---
S. spadiceum Fr.	White	3	++++	Tr.–20	+++	25–40	6
S. sulcatum Burt	White pocket.	1	+++++	0	+++++	10	5
S. umbrinum Berk. and Curt.	White	1	[2]− to +	35	+++	25	7
Hydnaceae:							
Echinodontium tinctorum Ell. and Ev.	do	5	++++	0	+++	Tr.	4
Hydnum caput-ursi Fr.	do	2	++++	0	++	0	4
H. corolloides Scop.	do	2	++++	0	++	0	4
H. erinaceus Bull.	do	20	++++	0	[4]− to +++	0–tr.	4
H. ochraceum Pers.	do	1	+++	0	++	0	4
H. pulcherrimum Berk. and Curt.		2	------	65–80	------	0	3
H. septentrionale Fr.	White	4	++	0	[4]++++	Tr.	4

See footnotes at end of table.

TABLE 1.—*Reaction and growth of wood-decaying fungi on gallic and tannic acid media, at the end of 7 days*—Continued

Fungus	Type of decay	Isolations	On gallic acid medium		On tannic acid medium		Growth and reaction group No.
			Reaction	Growth	Reaction	Growth	
		Number		*Mm*		*Mm*	
Hydnaceae—Continued.							
Irpex cinnamomeus Fr.	White	3	++++	Tr.-10	+++	25-35	6
I. mollis Berk. and Curt.	do	6	+++	0	+++	20-30	6
Phlebia strigosazonata Schw.	do	3	+++++	0	++++	20-30	6
Radulum orbiculare Fr.	do	1	++++	0	+++	Tr.	4
Polyporaceae:							
Daedalea ambigua Berk.	do	3	+++++	0	++++	15-20	5
D. confragosa (Bolt.) Fr.	do	5	++++	0	+++++	10-30	5
D. juniperina Murr.	Brown	5	-------	20	------	Tr.	3
D. quercina (L.) Fr.	do	13	–	20-30	–	20-30	1
D. unicolor (Bull.) Fr.	White	3	+++	0	+++	20-35	6
Favolus canadensis Klotz.	do	2	++++	0	++++	10	5
Fistulina hepatica (Huds.) Fr.	Dark firm.	15	–	15-20	–	10-25	1
Fomes annosus (Fr.) Cooke.	White	5	++++	0-tr.	+++	Tr.-10	5
F. applanatus (Pers.) Gill.	do	22	+++	0-10	++++	10-20	5
F. calkinsii (Murr.) Sacc. and Sacc. D.	do	4	++++	0-tr.	++++	10	5
F. conchatus (Pers.) Gill.	do	9	+++	0-tr.	+++	Tr.	4
F. connatus (Weinm.) Gill.	do	2	++	0	[4]+	0	4
F. densus Lloyd.	do	4	+++	Tr.-10	+++	0-tr.	7
F. everhartii (Ell. and Gall.) Schrenk.	do	26	++++	0	++++	10-15	5
F. fomentarius (L.) Gill.	do	12	+++	0	++++	10-20	5
F. fraxineus (Bull.) Cooke.	do	2	+++	0	++++	15-20	5
F. fraxinophilus (Peck) Sacc.	do	9	++++	Tr.-10	[4]+++	Tr.-15	7
F. fulvus (Scop.) Gill.	do	1	++++	Tr.	+++	25	5
F. geotropus Cooke.	do	2	[2]– to +++	35-40	[2]– to +	10-15	9
F. igniarius (L.) Gill.	do	6	++	0	+++	Tr.	4
F. igniarius var. *laevigatus* (Fr.) Overh.	do	4	+++	0-15	++++	Tr.-20	5
F. igniarius var. *populinus* (Neu.) Campb.	do	6	+++	Tr.-10	+++	Tr.	4
F. lobatus (Schw.) Cooke.	do	6	+++	0-10	+++	10-30	5
F. marmoratus Berk. and Curt.	do	2	++++	0	++++	10-15	5
F. meliae (Underw.) Murr.	Brown	2	–	20-45	–	15-30	1
F. officinalis (Vill.) Faull.	do	2	[5]–to+	10	–	0	---
F. ohiensis (Berk.) Murr.	White	1	+++++	0	++++	0	4
F. pini (Thore) Lloyd.	White pocket.	13	++++	Tr.-15	+++	Tr.-15	7
F. pinicola (Sw.) Cooke.	Brown	26	–	15-30	–	10-20	1
F. rimosus Berk.	White	10	++	0-10	++	0-tr.	4
F. robustus Karst.	do	6	++++	Tr.	++++	10-25	5
F. robustus var. *tsugina* (Murr.) Overh.	do	3	+++++	0-tr.	++++	Tr.	4
F. roseus (Alb. and Schw.) Cooke.	Brown	11	[5]–to++	15-25	[5]–to+	Tr.-15	---
F. scutellatus (Schw.) Cooke.	White	4	++++	0	+++	0-15	5
F. subroseus (Weir) Overh.	Brown	3	[5]–to+	25-30	–	10-25	---
F. tenuis Karst.	White pocket.	3	+++++	0	++++	Tr.-10	5
Sterile *Fomes* on birch.	White	13	+++	0	+++	Tr.-15	5
Lenzites betulina (L.) Fr.	do	3	++++	Tr.	+++	30-45	6
L. saepiaria (Wulf.) Fr.	Brown	6	–	10-15	–	Tr.	3
L. striata (Sw.) Fr.	do	1	–	25	–	10	2
L. trabea (Pers.) Fr.	do	4	–	20-30	–	0-tr.	3
Merulius confluens Schw.	White	1	[2]+	20	–	0	9
M. tremellosus (Schrad.) Fr.	do	7	+++++	0-40	++++	20-40	7
Polyporus abietinus (Dicks.) Fr.	White pocket.	1	++++	0	++++	Tr.	4
P. adustus (Willd.) Fr.	White	6	[2]–to++	20-40	+	15-25	7
P. albellus Peck.	do	6	+++++	0	++++	Tr.-10	5
P. alboluteus Ell. and Ev.	White [6]	1	+++	55	+++	60	7
P. amygdalinus Berk. and Rav.	Brown	2	–	40	–	40	1
P. anceps Peck.	White	10	+++++	0-tr.	[4]++++	Tr.-25	5
P. arcularius (Batsch.) Fr.	---	1	++++	Tr.	+++	35	6
P. balsameus Peck.	Brown	11	[5]–to+	15-25	–	0-tr.	---
P. berkeleyi Fr.	White	9	++++	0	++++	0-10	4
P. betulinus (Bull.) Fr.	Brown	4	–	35	–	15-20	2
P. borealis Fr.	White	3	++++	0	+++	0-tr.	4
P. brumalis (Pers.) Fr.	do	4	++++	0	++++	20-30	6
P. cinnabarinus (Jacq.) Fr.	do	8	+++++	0-tr.	++++	20-30	6
P. circinatus Fr.	do	5	++++	0-tr.	+	0-tr.	4
P. compactus Overh.	do	11	++++	15-30	+++	20-40	7

See footnotes at end of table.

TABLE 1.—*Reaction and growth of wood-decaying fungi on gallic and tannic acid media, at the end of 7 days*—Continued

Fungus	Type of decay	Isolation number	On gallic acid medium		On tannic acid medium		Growth and reaction group No.
			Reaction	Growth	Reaction	Growth	
Polyporaceae—Continued.				*Mm*		*Mm*	
P. conchifer (Schw.) Fr	White	1	++++	0	++++	15	5
P. croceus (Pers.) Fr	do	17	++++	10–35	++	20–40	7
P. curtisii Berk	do	3	++++	10–20	+++	15–30	7
P. cuticularis (Bull.) Fr	do	14	+++	0	++++	Tr.–15	5
P delectans Peck	do	4	+++	0	+++	Tr.–10	5
P. dichrous Fr	do	4	−	10	−	0	3
P. distortus (Schw.) Fr	do	4	++++	10–15	+++	30–40	8
P. dryadeus (Pers.) Fr	do	2	+++	0	+++	0	4
P. dryophilus Berk	do	17	++++	10–25	+++	10–25	7
P. fibrillosus Karst	Brown	1	−	15	−	15	1
P. fissilis Berk and Curt	White	12	++++	0	+++	Tr.–10	5
P. fragilis Fr	Brown	4	−	10	−	0	3
P. frondosus (Dicks.) Fr	White	10	+++	15–35	+++	10–35	7
P. fumidiceps (Atk.) Sacc. and Trott.	do	1	++++	Tr.	++++	20	5
P. fumosus (Pers.) Fr	do	2	[2]−to+	15–30	[2]−to+	Tr.–15	7
P. galactinus Berk	do	7	+++++	0–tr.	++++	Tr.–15	5
P. giganteus (Pers.) Fr	do	4	+++	0	++++	10	5
P. gilvus (Schw.) Fr	do	11	+++++	Tr.–15	+++	Tr.–30	5
P. glomeratus Peck	do	10	+++	0	+++	0	4
P. graveolens (Schw.) Fr	do	8	[4]++	0	[4]+++	Tr.–20	5
P. guttulatus Peck	Brown	2	−	15–25	−	0	3
P. helveolus Rostk		1	−	40	−	20	2
P. hirsutus (Wulf.) Fr	White	7	+++++	0	+++	20–40	6
P. hispidus (Bull.) Fr	do	15	++++	0–10	++++	10–20	5
P. immitus Peck	Brown	3	−	20	−	0	3
P. lucidus (Curt.) Fr	White	14	++++	0–30	+++	15–40	7
P ludovicianus (Pat.) Sacc. and Trott.	White pocket.	6	+++	Tr.–10	+++	Tr.	9
P. mutabilis Berk and Curt	White	2	+++	0	+	0	4
P. obtusus Berk	do	11	++++	0	++++	Tr.–15	5
P. oregonensis (Murr.) Kauff	do	2	++++	0	++++	10	5
P. osseus Kalchbr		1	−	55	−	0	3
P. palustris Berk and Curt	Brown	2	−	15–30	−	15–20	1
P. pargamenus Fr	White	4	++++	0	++++	Tr.–15	5
P. picipes Fr	do	1	++	0	+	0	4
P. pubescens (Schum.) Fr	do	4	+++++	0	+++	30–40	6
P. radiatus (Sow.) Fr	do	9	+++	Tr.–10	+++	Tr.–15	7
P. resinosus (Schrad.) Fr	do	9	++++	Tr.–40	+++	10–35	7
P. robiniophilus (Murr.) Lloyd	do	5	+++	0	+++	Tr.–20	5
P. sericeo-mollis Rom	Brown	7	−	20–35	−	Tr.–10	3
P. schweinitzii Fr	do	9	−	15–35	−	0	3
P. spraguei B. and C	do	27	−	30–45	−	20–40	1
P. spumeus (Sow.) Horne	White	3	+++	0–tr.	++++	Tr.–20	5
P. squamosus (Huds.) Fr	do	3	+++	0	+	0	4
P. subertypus (Murr.) Overh. in comb.	do	1	++++	0	+++	35	6
P. sulphureus (Bull.) Fr	Brown	23	−	25–45	−	20–45	1
P. supinus (Sw.) Fr	White	7	+++	0	++++	15–30	5
P. tenuis (Sacc.) Overh	do	2	++++	0	+++	40–50	6
P. tephroleucus Fr	Brown	2	[7]−	15	−	0	3
P. tsugae (Murr.) Overh	White	5	+++	0–tr.	+++	Tr.–25	5
P. tuberaster (Jacq.) Fr		2	+++	0	+++	0–tr.	4
P. tulipiferus (Schw.) Overh	White	9	[2]−to++	40–60	[2]−to+	Tr.–30	9
P. umbellatus (Pers.) Fr		1	+++	0	+	0	4
P. velutinus Fr	White	1	++++	0	+++	40	6
P. versicolor (L.) Fr	do	6	+++++	0	+++	30–50	6
P. volvatus Peck	do	3	+++	0	++++	Tr.–10	5
P. vulpinus Fr	do	1	+++	Tr.	+	0	4
P. zonalis Berk	White pocket.	5	++	0	[4]+++	Tr.–25	5
Poria andersonii (E. and E.) Neuman.	White	11	++++	0	++++	Tr.–10	5
P. borbonica Pat	do	1	++++	10	+++	60	8
P. cocos (Schw.) Wolf	Brown	12	[5]−to+	50–80	−	30–80	
P. incrassata (Berk. and Curt.) Burt.	do	4	−	20–40	−	0–tr.	3
P. medulla panis (Pers.) Cooke	White	2	+++++	Tr.–15	+++	30	8
P. mutans Peck	do	1	++++	20	+++	20	7
P. mutans var. tenuis Peck	do	1	++++	20	+++	30	7
P. nigra Berk	Brown	3	−	25–35	−	25–35	1
P. nigrescens Bres	White	3	++++	0	++++	Tr.–10	5

See footnotes at end of table.

TABLE 1.—*Reaction and growth of wood-decaying fungi on gallic and tannic acid media, at the end of 7 days*—Continued

Fungus	Type of decay	Isolations	On gallic acid medium		On tannic acid medium		Growth and reaction group No.
			Reaction	Growth	Reaction	Growth	
		Number		*Mm*		*Mm*	
Polyporaceae—Continued.							
P. prunicola (Murr.) Sacc. and Trott.	White	5	++++	Tr.–15	+++	Tr.–20	7
P. pulchella (Schw.) Cooke	do	5	+++++	Tr.–15	+++	15–50	8
P. punctata Fr	do	6	+++++	0-tr.	++++	10–20	5
P. selecta Karst. ex Rom	Brown	1	–	45	–	Tr.	3
P. subacida (Peck) Sacc	White	14	++++	0–10	+++	10–30	8
P. undata (Pers.) Bres	White pocket	3	++	0	+++	10–20	5
P. vaillantii Fr	Brown	1	–	25	–	0	3
P. vaporaria Fr	Brown [6]	2	–	30	–	0–10	2
P. versipora Pers	White	1	++++	0	++++	15	5
P. weirii (Murr.) Sacc. and Trott	do	2	+++	20–30	+++	20–30	7
Trametes americana Overh	Brown	2	–	10–15	–	Tr.	3
T. cubensis (Mont.) Sacc		1	–	70	–	0	3
T. heteromorpha (Fr.) Lloyd	Brown	1	[5]–to+	20	–	0	
T. hispida Bagl	White	1	++++	0	++++	25	5
T. hydnoides (Sw.) Fr	do	1	[2]–to++	25	+	10	9
T. malicola B. and C	Brown	2	–	15	–	Tr.–10	1
T. mollis (Som.) Fr	White	2	+++	0	++++	Tr.	4
T. rigida Berk. and Mont	do	1	+++	0	++++	20	5
T. serialis Fr	Brown	5	[7]–	15–40	–	10–30	1
T. suaveolens Fr	White	3	++++	0	+++	10–30	5
Agaricaceae:							
Armillaria mellea (Vahl) Quél	do	12	++++	0-tr.	++++	Tr.–10	5
Clitocybe illudens (Schw.) Sacc	do	1	++++	10	++++	15	7
C. tabescens (Scop.) Bres	do	6	++++	0–10	++++	Tr.–10	7
Collybia velutipes (Curt.) Quél	do	4	+	0	–	0	4
Hypholoma sublateritium Schaeff	do	6	++++	0-tr.	++++	Tr.–20	5
Lentinus lepideus Fr	Brown	8	[5]–to+++	Tr.–15	[5]–to+	0-tr.	
L. tigrinus (Bull.) Fr	White	4	++++	Tr.–25	+++	35–45	8
Panus laevis Berk. and Curt	do	4	+++	0	++	0-tr.	4
P. stipticus (Bull.) Fr	do	4	++++	0-tr.	++++	Tr.–20	5
Pholiota adiposa (Pers.) Fr	do	8	+++	0	+++	Tr.–20	5
Pleurotus corticatus Fr	do	4	+++	0	+++	0-tr.	4
P. ostreatus (Jacq.) Quél	do	11	+++	0	[4]+++	Tr.–10	5
P. serotinus	do	2	+++	0–20	+++	Tr.–30	7
P. ulmarius (Bull.) Quél	do	6	–to+	0	–	0	4
Schizophyllum commune Fr	do	4	–	20–40	[2]++	25–40	10
Ascomycetes:							
Ustulina vulgaris Tul	do	7	+++++	Tr.–20	+++	20–25	8
Xylaria sp.	do	2	+++++	Tr.	+++	30–40	6
Fungi Imperfecti:							
Strumella coryneoides Sacc. and Wint.	do	6	+++++	20–25	+++	30–50	8

[1] Growth and reaction groups are explained in detail in the text.

[2] These fungi may require from 7 to 14 days to form brown diffusion zones, but the reactions are always positive.

[3] Reaction uncertain; most isolations formed a brown diffusion zone under mat in 7 days. In 14 days zone more intense but certain isolations are consistently negative.

[4] Reaction positive if growth occurs; if no growth, usually positive but occasionally negative.

[5] Reaction usually negative. However, a faint brown diffusion zone may form under center of mat. The same isolation may be negative at one trial but slightly positive at another.

[6] SHOPE, PAUL FRANKLIN. THE POLYPORACEAE. OF COLORADO. Ann. Mo. Bot. Gard. 18: 287–456, illus. 1931.

[7] Reaction negative. A widely diffused light-brown indefinite zone may appear around but not under mat. Agar under mat perfectly transparent.

NEGATIVE OR NONREACTING FUNGI

Group 1.—Mat diameter for a given species about equal on both gallic and tannic acid media. 14 fungi. (Fig. 1, *A* and *B*.)

Group 2.—Growth on gallic acid medium, good, mat diameter much greater than on tannic acid medium. Five fungi. (Fig. 1, *C* and *D*.)

Group 3.—Good growth on gallic acid medium, none or only a trace on tannic acid medium. 17 fungi. (Fig. 1, *E* and *F*.)

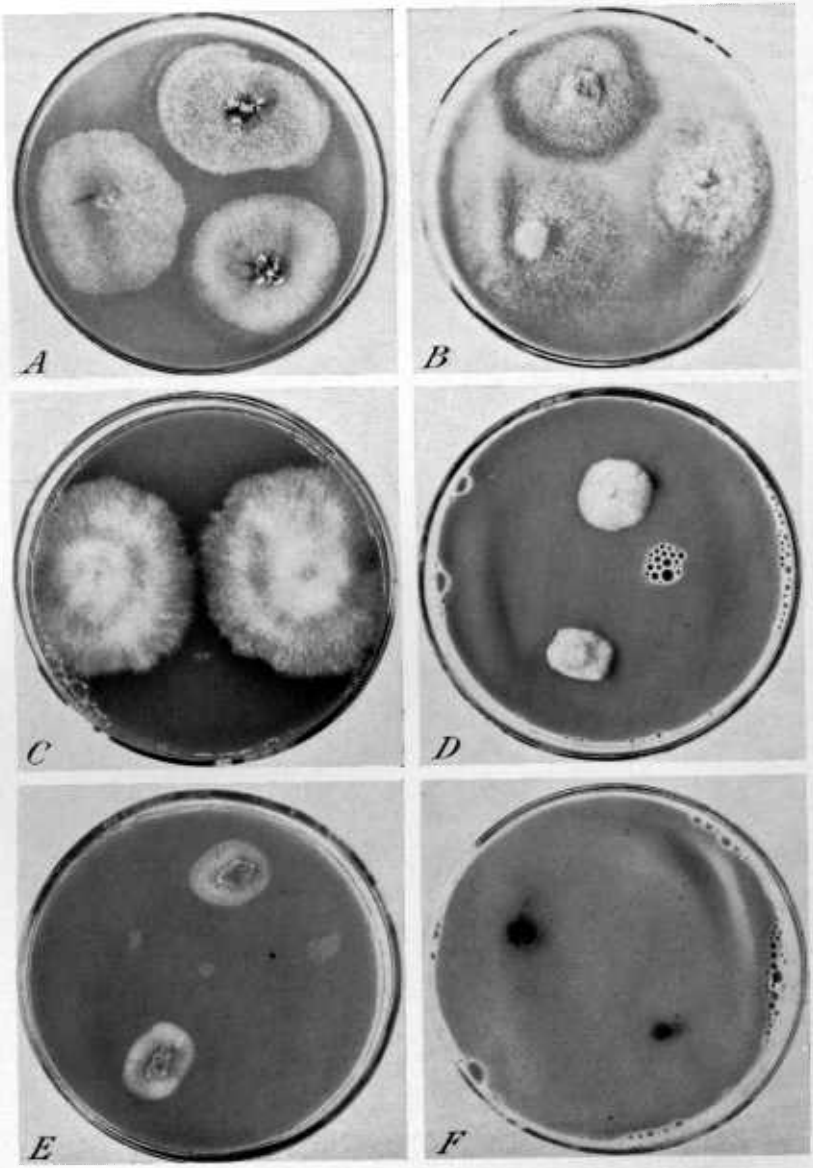

FIGURE 1.—Negative or nonreacting groups. *A* and *B*, Group 1 as illustrated by *Polyporus sulphureus;* mat diameters for a given species approximately the same on gallic (*A*) and tannic (*B*) acid media. *C* and *D*, Group 2, as illustrated by *Coniophora suffocata;* good growth on gallic (*C*) acid medium, poor on tannic (*D*) acid medium. *E* and *F*, Group 3 as illustrated by *Daedalea juniperina;* good growth on gallic (*E*) acid medium, no growth or only a trace on tannic (*F*) acid medium.

POSITIVE-REACTING FUNGI

Group 4.—No growth, or only a trace, on both gallic and tannic acid media. 34 fungi. (Fig. 2, *A* and *B*.)

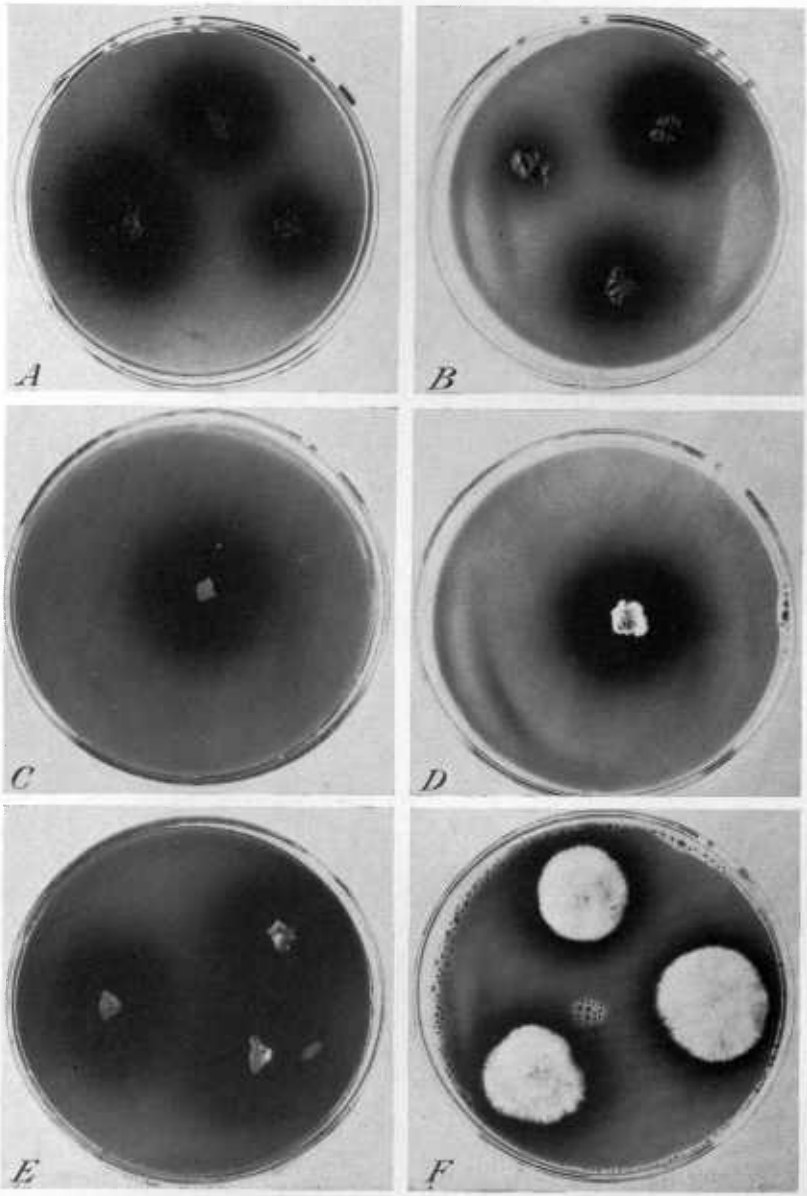

FIGURE 2.—Positive-reacting groups. *A* and *B*, Group 4 as illustrated by *Polyporus glomeratus;* no growth or only a trace on gallic (*A*) and tannic (*B*) acid media. *C* and *D*, Group 5 as illustrated by *Stereum murrayi;* no growth or only a trace on gallic (*C*) acid medium, trace to 25 mm on tannic (*D*) acid medium. *E* and *F*, Group 6 as illustrated by *Irpex mollis;* no growth or only a trace on gallic (*E*) acid medium, growth 25–50 mm on tannic (*F*) acid medium.

Group 5.—No growth or only a trace on gallic acid medium, mat diameter 25 mm or less on tannic acid. 57 fungi, all strong reactors. (Fig. 2, *C* and *D*.)

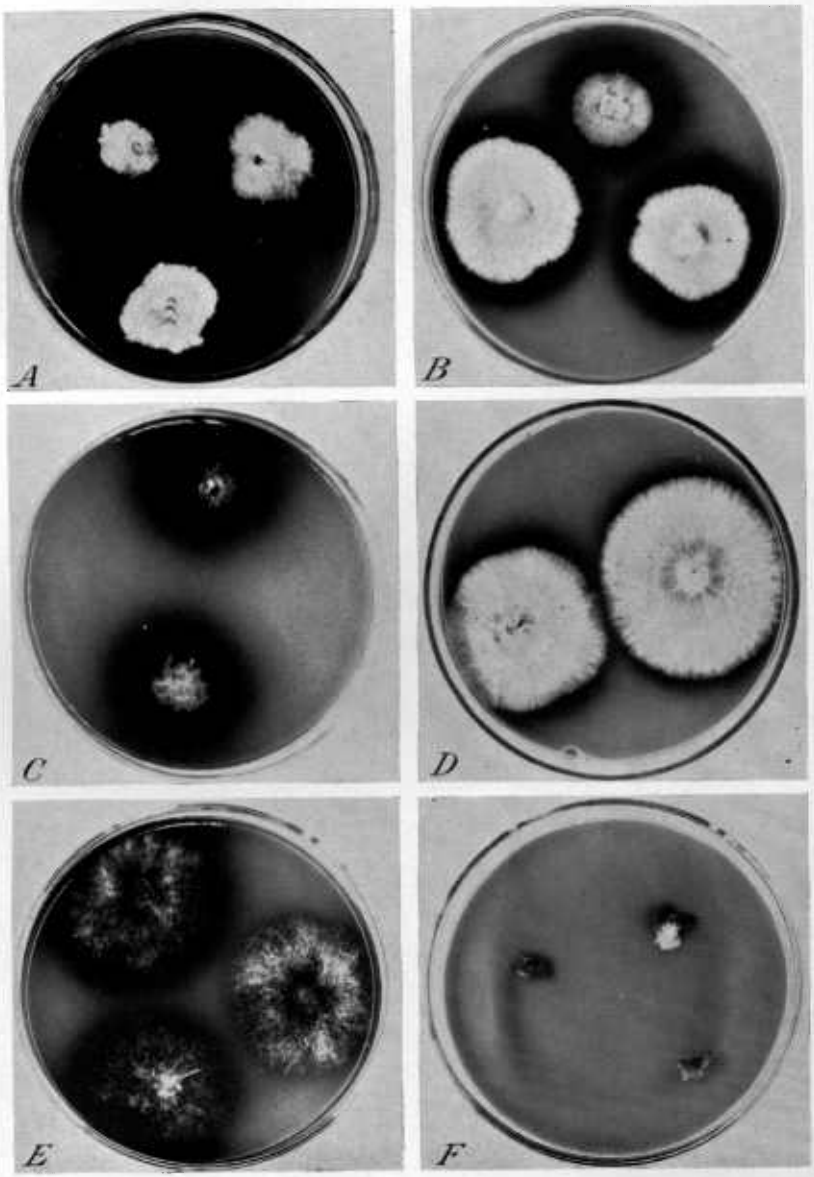

FIGURE 3.—Positive-reacting groups. *A* and *B*, Group 7 as illustrated by *Polyporus compactus;* mat diameters approximately equal on both gallic (*A*) and tannic (*B*) acid media. *C* and *D*, Group 8 as illustrated by *Polyporus distortus;* slight growth on gallis (*C*) acid medium, good growth on tannic (*D*) acid medium. *E* and *F*, Group 9 as illustrated by *Polyporus tulipiferus;* good growth on gallic (*E*) acid medium, none to a trace on tannic (*F*) acid medium.

Group 6.—Growth none or only a trace on gallic acid medium, growth 25–50 mm on tannic acid. 22 fungi. (Fig. 2, *E* and *F*.)

Group 7.—Mat diameters about equal on both media. 31 fungi. (Fig. 3, *A* and *B*.)

Group 8.—Fair growth on gallic acid medium, good growth on tannic acid medium. 12 fungi. (Fig. 3, *C* and *D*.)

Group 9.—Good growth on gallic acid medium, none to trace on tannic acid medium, often weak reactors, some requiring 14 days for definite results. Nine fungi. (Fig. 3, *E* and *F*.)

FUNGI HAVING NEGATIVE OR POSITIVE REACTION, DEPENDING ON MEDIUM

Group 10.—Reaction negative on gallic acid medium, positive on tannic acid medium, with good growth on both. One fungus (*Schizophyllum commune*).

DISCUSSION

Naturally, since arbitrary limits have been prescribed for the above-mentioned groups, certain fungi should be expected to show growth behavior which may fall within two or more groups. Each fungus is placed in a group on the basis of its most consistent and characteristic growth behavior, and the relative consistency with which different isolates fall into a given group may vary for the different species. For this reason, in comparing growth groups of two different species, more significance should be attached to growth reactions involving widely separated groups such as groups 4 and 9 than to those involving closely related groups such as 4 and 5 or 5 and 6. This applies to both positive and negative reactors. In order to demonstrate the consistency with which a given fungus would fall into a definite group on repeated trials of the same isolate, or with a number of isolates, the data for a few fungi, for which several isolates were available, have been analyzed. Ninety-five percent of the recorded conclusive trials place *Polyporus compactus* in group 7 and 5 percent in group 6 (21 trials); 54 percent place *P. cuticularis* in group 5 and 46 percent in group 4 (22 trials); 73 percent place *P. dryophilus* in group 7, 21 percent in group 9, and 5 percent in group 4 (19 trials); 82 percent place *P. hispidus* in group 5 and 17 percent in group 4 (17 trials); 97 percent place *P. spraguei* in group 1 and 3 percent in group 3 (33 trials); 73 percent place *P. supinus* in group 5 and 27 percent in group 6 (15 trials); and 72 percent place *Poria andersonii* in group 5 and 28 percent in group 4 (18 trials).

The foregoing growth and reaction groups are constant and distinct enough to be useful in the identification of certain fungi. For example, cultures of *Polyporus pargamenus* and *P. tulipiferus* are sufficiently similar to be confused in the laboratory. On 0.5-percent gallic and tannic acid media, however, the reaction of the former falls under group 5, that of the latter under group 9. The differences between the two species on these media cannot be confused. In many cases identification of isolations from unnamed sporophores may be assured if the type of decay associated with the sporophore can be determined. The oxidase test is useful in such cases as well as in the general determination of decay types for fungi taken from incipient decay before the typical stage manifests itself. *P. schweinitzii* and *P. circinatus* sporophores are similar, but cultures of the two fungi can be readily separated on gallic and tannic acid media, as the former is a nonreactor while the latter is a positive reactor.

A number of other instances where the oxidase test has been of value could be cited, but the few listed here give a general idea of cases in which the oxidase reaction, correlated with other information, has been of particular value.

A column showing type of decay is included in table 1 to give some idea of the kind of rot that is usually associated with each fungus. The type of rot has not been determined by pure-culture methods but by the association of sporophores with the rots in question. In many cases, as in certain groups of the Thelephoraceae, where no definite association of rot and fungus has been found, the space is left blank.

As used in the paper, the term "brown rot" refers only to the brown cubical type of decay. Most of the others are classified as white rots, although many are somewhat colored owing to the color of the wood or to changes associated with incipient stages of infection. For instance, *Stereum frustulosum*, *S. subpileatum*, and *Hydnum erinaceus* are usually present in brown-stained wood, but the wood is white or light-colored in the final stages of decay.

Of the 210 fungi that were tested for the presence of oxidases 36 were negative, i. e., they did not form brown diffusion zones on either medium; 8 were inconsistent, i. e., they were usually negative, but certain isolations gave slight brown diffusion zones on one or both media; 165 were positive; and 1 (*Schizophyllum commune*) gave consistently negative results with gallic acid medium but positive results with tannic acid, with good growth on both.

Of the 36 fungi that were consistently negative, 29 are associated with brown carbonizing rots;[7] the type of decay for 4 fungi could not be checked. *Fistulina hepatica* causes a darkening of the wood but not a typical brown rot; *Stereum frustulosum* causes a white pocket rot, which produces dark discoloring of the wood about the decayed pockets; and *Polyporus dichrous* is associated with what appears to be a definite white rot. Of the eight fungi giving inconsistent, but mainly negative results, seven are definitely brown rot fungi while *S. subpileatum* is associated with a white pocket rot with darkening of the surrounding wood.

Of the 165 fungi giving consistently positive oxidase reactions, 156 are associated with white rot;[8] the type of decay was unknown for 9 species. *Schizophyllum commune*, associated with a white decay, was negative on gallic acid but positive on tannic acid medium, with good growth on both.

Eighty percent of the negative reactors were correctly diagnosed as brown rot fungi and 96 percent of the positive reactors were cor-

[7] The majority of brown rot fungi showed no signs of reaction on either gallic or tannic acid medium. This was especially true of those species producing white mycelia in culture. The species that gave inconsistent results usually formed yellow, buff, or brown mycelia in culture, and it was often difficult to decide whether the faint discoloration, as seen from the under side of the Petri dish, was due to the color of the mat or to reaction with the medium. This was especially true on the transparent gallic acid medium.

[8] White rot fungi showed considerable variation in the intensity of the brown diffusion zone and in the time required for its formation. Very strong reactors gave a definite zone in 1 or 2 days. The great majority produced definite results in 7 days while only a few fungi required a longer time for the zone to form. Strongest reactors gave positive results with little or no growth, especially on gallic acid. A few gave results only with growing mycelium. For this reason tests showing no reaction and no growth on either medium were considered inconclusive, but tests showing a reaction without growth were considered conclusive. Since all fungi do not react equally well with both tannic and gallic acid media both should be used when the purpose of the test is to determine decay type by the oxidase reaction.

rectly diagnosed as white rot fungi. Thus Bavendamm's generalization, that brown rot fungi are negative when tested for oxidases by the use of gallic and tannic acids and that white rot fungi give positive reactions with the same media is essentially correct.

SUMMARY

Two hundred and ten fungi were tested for oxidases, using Bavendamm's gallic and tannic acid method.

Twenty-nine, or 80 percent, of the fungi associated with brown carbonizing rots gave no reaction with gallic or tannic acid media. One (*Fistulina hepatica*), associated with a dark firm rot, was negative, as was *Stereum frustulosum*, associated with a white pocket rot, and *Polyporus dichrous*, associated with a white rot. Four whose rots were unknown gave no reaction. Seven fungi associated with brown carbonizing rots gave inconsistent reactions.

One hundred and fifty-six, or 96 percent, of those fungi associated with white rots gave positive reactions. Nine specimens whose rots were unknown also gave positive reactions. *Stereum subpileatum*, associated with a white pocket rot similar to *S. frustulosum*, gave mostly positive results on gallic acid but was negative on tannic acid. *Schizophyllum commune*, a white rot fungus, was negative on gallic acid medium but positive on tannic acid medium, with good growth on both media.

With white rot fungi the intensity of reaction and the time required for the reaction to take place showed considerable variation.

Growth behavior for different species on gallic acid and tannic acid media showed variations of value in the cultural identification of fungi. Ten different growth and reaction groups are described and the species are listed under these groups.